PATENT COOPERATION TREATY

PCT

09/269607

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
PAT97013*PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 97/02267	21/08/1997	21/02/1997
Applicant		
NOKIA MOBILE PHONES LTD et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 2 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [ ] the text is approved as submitted by the applicant
   - [X] the text has been established by this Authority to read as follows:

   AN ERGONOMICALLY SHAPED HANDSET

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. 4
   - [X] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/02267

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 H04M1/02 H05K5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 H04M H04B H05K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 332 322 A (GAMBARO THOMAS L) 26 July 1994 see abstract; figures	1-5, 12-15
A	US 5 383 091 A (SNELL RUSTY B) 17 January 1995 see abstract; figures	1,13

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
5 December 1997	15/12/1997

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Goulding, C

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 97/02267

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5332322 A	26-07-94	US 5178477 A	12-01-93
US 5383091 A	17-01-95	NONE	

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : G01N 33/68

A1

(11) International Publication Number: WO 98/13694

(43) International Publication Date: 2 April 1998 (02.04.98)

(21) International Application Number: PCT/GB97/02667

(22) International Filing Date: 29 September 1997 (29.09.97)

(30) Priority Data:
9620195.9 27 September 1996 (27.09.96) GB

(71) Applicant (*for all designated States except US*): KING'S COLLEGE [GB/GB]; Strand, London WC2R 2LS (GB).

(72) Inventor; and
(75) Inventor/Applicant (*for US only*): EBRINGER, Alan [GB/GB]; 76 Gordon Road, Ealing, London W5 2AR (GB).

(74) Agents: POWELL, Stephen, David et al.; Williams, Powell & Associates, 4 st. Paul's Churchyard, London EC4M 8AY (GB).

(81) Designated States: JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: DIAGNOSIS OF SPONGIFORM DISEASE

(57) Abstract

A diagnostic test is provided for spongiform encephalopathy and other demyelinating conditions in mammals which comprises assaying antibodies present in the mammal which bind to an antigenic peptide which exhibits molecular mimicry of a mammalian myelin peptide, e.g. one having the sequence FSWGAEGQK. This test is useful for detecting BSE in cattle by assaying sera collected from the cattle for antibodies to a species of Acinetobacter, Agrobacterium or Ruminococcus, or a peptide having a sequence present in said species which mimics a peptide of bovine myelin and identifying animals having a level of antibodies at least about two standard deviations above that of healthy control animals.

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Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

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DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

INTERNATIONAL SEARCH REPORT

Imerr ...tal Application No
PCT/GB 97/02667

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/68

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 80, no. 11, 18 March 1974 Columbus, Ohio, US; abstract no. 56313, A. WAJGT.: "Assessment by immunofluorescence methods of humoral antimyelin antibody in rats with cyanide encephalopathy." page 68; column 1; XP002052988 see abstract & ANN. IMMUNOL. (POZNAN), vol. 5, no. 1-2, 1973, pages 51-58.	1

-/—

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

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"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
22 January 1998	04/02/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Griffith, G

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/GB 97/02667

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	B. H. TOH ET AL.: "The 200- and 150-kDa neurofilament proteins react with IgG autoantibodies from patients with kuru, Creutzfeldt-Jakob disease, and other neurologic diseases." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA., vol. 82, May 1985, WASHINGTON US, pages 3485-3489, XP002052986 ---	
A	R. L. SIDMAN ET AL.: "Transmissible spongiform encephalopathy in the gray tremor mutant mouse." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA., vol. 82, January 1985, WASHINGTON US, pages 253-257, XP002052987 ---	
A	CHEMICAL ABSTRACTS, vol. 109, no. 21, 21 November 1988 Columbus, Ohio, US; abstract no. 187890, M. P. MCKINLEY ET AL.: "Developmental regulation of prion protein mRNA in brain." page 484; column 2; XP002052989 see abstract & CIBA FOUND. SYMP., vol. 135(Novel Infect. Agents Cent. Nerv. Syst.), 1988, pages 101-116. ----	

PATENT COOPERATION TREATY

09/269607

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

POWELL, Stephen David
Williams, Powell & Associates
4 St. Paul's Churchyard
London EC4M 8AY
GRANDE BRETAGNE

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing (day/month/year)	11. 01. 99
Applicant's or agent's file reference: SP/N6195	IMPORTANT NOTIFICATION

International application No.	International filing date (day/month/year)	Priority date (day/month/year)
PCT/GB97/02667	29/09/1997	27/09/1996

Applicant
KING'S COLLEGE et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/
European Patent Office
D-80298 Munich
Tel. (+49-89) 2399-0, Tx: 523656 epmu d
Fax: (+49-89) 2399-4465

Authorized officer
Hébert, W
Tel. (+49-89) 2399-8161

09/269607

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (PCT/IPEA/416)
SP/N6195		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB97/02667	29/09/1997	27/09/1996
International Patent Classification (IPC) or national classification and IPC		
G01N33/68		
Applicant		
KING'S COLLEGE et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☒	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☒	Certain defects in the international application
VIII	☒	Certain observations on the International application

Date of submission of the demand	Date of completion of this report
24/04/1998	
Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Thiele, U Telephone No. (+49-89) 2399-8643

Form PCT/IPEA/409 (Cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB97/02667

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-6 as originally filed

Claims, No.:

1-12 as originally filed

Drawings, sheets:

1/1 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 1-4,5(part)-8(part),9-12.

because:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB97/02667

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☒ the claims, or said claims Nos. 1-4,5(part)-8(part),9-12 are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	5-8
	No:	Claims	
Inventive step (IS)	Yes:	Claims	5-8
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	5-8
	No:	Claims	

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB97/02667

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/GB97/02667

Section III

1) The subject-matter of claims.2, 10 and 12 is not supported by the description (Art. 6 PCT).

It is apparent from the instant specification, see the table on page 2, that the mammalian myelin peptide sequence which is similar to microbial sequences is "LSRFSWGAE" rather than "FSWGAEGQK". It is considered that at least the "R" which precedes "FSWGAE" is essential for provoking cross reactivity with the Acinetobacter caloaceticus sequence as substantiated in the examples and thus for molecular mimicry.

The applicant, in response to the Written Opinion has submitted a copy of the inventor's scientific publication "Environmental Health Perspectives 105, 1, Nov. 1997, 1172 - 1174" (D2) which has colour space filling models of myelin, bacterial antigen and prion structures to assist visualisation. The IEA interprets Figure 1 of said document such that it emphasizes the importance of residues "R", "W" and "E" for cross reactivity. Consequently, the afore made objection is fully maintained.

2) The subject-matter of claims 1 - 12 represents an inadmissible generalization from particular examples (Art. 6 PCT).

a) The applicant in the present description, page 1, bottom line, refers to the sequence "FSWGAEGQK" as being "in **denatured** form [...] encephalitogenic" (emphasis added). A denatured protein does not adopt the native three-dimensional structure.

It is self-evident that cross reactivity, let alone cross reactivity as regards a denatured protein sequence, cannot be predicted a priori on the basis of mere sequence similarities. Cross reactivity is i.a. dependent on the epitope sequence, conformation and charge distribution. The latter two aspects appear to be illustrated even by document D2, Figures 1 and 2.

Please note that the Ruminococcus albus sequence (see page 2) has even not

more than three amino acids in common with the bovine myelin sequence.

In conclusion, on the basis of one particular example (Acinetobacter caloaceticus) in the present application and the further example in D2 (Agrobacterium; see Figure 1) it cannot be reasonable expected that the further sequences cited in present claim 1 would have the same effects.

b) In view of the up to now unclear molecular mechanisms (see present application, page 1, paragraph 3) which are responsible for such different diseases as BSE and Multiple Sclerosis, it is not reasonable to expect that the concept exemplified for BSE in the present application could be applied to further forms of spongiform encephalopathy and demyelating conditions (claims 1 and 2) without undue experimentation by using merely routine methods. It is worth while noting that the starting point for the present invention as regards BSE has been the long known peptide "FSWGAEGQK" (see present application, page 1). This peptide has been used to search databases for similar sequences (see D2, page 1173, r. col., last sentence; see present application, page 2, first paragraph). No such critical peptides appear to be known for the other pathological conditions mentioned.

c) It is not reasonable to expect that the sequences found in certain species of Acinetobacter, Agrobacterium and Ruminococcus are conserved in all members of the respective genera. There appears to be a basis solely for the effect produced on account of the Acinetobacter calcoaceticus and the Agrobacterium tumefaciens sequence (see present application; see D2).

Section V

1) Reference is made to the following document:

D1 Proc. Natl. Acad. Sci. USA 82, 1985, 3485 - 3489

2) Notwithstanding the afore-made objections, the subject-matter of claims 5 - 8, insofar as it refers to the diagnosis of BSE with the aid of Acinetobacter calcoaceticus, Agrobacterium tumefaciens or the peptide "LSRFSWGAE", would

appear to be novel and inventive in view of the known state of the art (Art. 33(2), (3) PCT).

D1 merely speculates that enhanced production of autoantibody in patients with chronic neurologic diseases to neurofilaments may be triggered by infection with unconventional viruses as well as by other agents that provoke central nervous system degeneration. In other words, preexisting clones of antibody-producing cells are activated in these disease. In that context, D1 points to the cross-reaction that has been verified for measles virus protein and herpes simplex virus protein with vimentin (see page 3488, r. col., bottom paragraph et seq.).

The further documents cited in the ISR merely refer to distant state of the art.

There is, however, no indication in the prior art which would direct the skilled person to develop a diagnostic test for BSE, which test uses a particular peptide for assaying antibodies and wherein the peptide exhibits molecular mimicry of a mammalian myelin peptide .

Section VII

Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the document D1 is not mentioned in the description, nor is this document identified therein.

Section VIII

For the assessment of the present claims relating to Acinetobacter calcoaceticus on the question whether they are supported by the description (Art. 5 PCT), no unified criteria exist in the PCT. The patentability can depend on the date at which the deposited microorganisms referred to on page 4, lines 4 - 5, has been made available to the public.

INTERNATIONAL SEARCH REPORT

Interr ıal Application No
PCT/GB 97/02667

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/68

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimumdocumentation to the extent that such documents are included in the fields searched

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[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex

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"&" document member of the same patent family

Date of the actual completion of theinternational search	Date of mailing of the international search report
22 January 1998	04/02/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Griffith, G

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/GB 97/02667

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate of the relevant passages	Relevant to claim No
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A	CHEMICAL ABSTRACTS, vol. 109, no. 21, 21 November 1988 Columbus, Ohio, US; abstract no. 187890, M. P. MCKINLEY ET AL.: "Developmental regulation of prion protein mRNA in brain." page 484; column 2; XP002052989 see abstract & CIBA FOUND. SYMP., vol. 135(Novel Infect. Agents Cent. Nerv. Syst.), 1988, pages 101-116, -----	

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DIAGNOSIS OF SPONGIFORM DISEASE

This invention relates to the detection of spongiform encephalopathy and other demyelinating conditions in mammals and is particularly, but not exclusively, concerned with the diagnosis of bovine spongiform encephalopathy (BSE).

BSE is a recent neurological disorder of cattle, which was first reported in the U.K. after 1982, following a change in the preparation of "bone and meal" feeds. BSE has attracted some public concern, lest it be transmitted to humans following meat consumption. It has been suggested that BSE is caused by "prions", a type of infectious protein.

The present invention is based on an alternative model of the genesis of various forms of spongiform encephalopathy and other demyelinating conditions in mammals. According to the proposed model, BSE and related diseases are conceived as autoimmune diseases arising as a result of molecular mimicry between certain infective agents and the myelin of the infected mammal. This new model of BSE, in particular, is based on the following experimental observations.

A characteristic histopathological feature of BSE is a "spongiform" appearance, which also occurs in chronic but not acute "experimental allergic encephalomyelitis" (EAE), at least in rabbits and guinea pigs. A short sequence of bovine myelin (FSWGAEGQK), which withstands denaturation following heating to 100°C for one hour, was reported over twenty-five years ago to produce hind quarters paralysis, tremors and death, following inoculation into guinea pigs, which to some extent resembles the features observed in cattle suffering from BSE. In accordance with the present invention, this sequence has been used as a computer probe to search for proteins showing molecular mimicry. This sequence, in denatured form, may be described as encephalitogenic.

Analysis of proteins in databases (Genbank and SwissProt) revealed that 3 microbes showed molecular mimicry of the bovine myelin sequence , the best one being found in 4-carboxy-muconolactone-decarboxylase of Acinetobacter calcoaceticus, a common microbe present in soil and water supplies. These sequence similarities are shown in the following Table.

Comparison of amino acids of bovine myelin to microorganisms from Genbank and SwissProt which have similar sequences in other proteins.

Source	Amino acids	Positions	Locations
Bovine myelin	LSRFSWGAE	110 - 118	
Acinetobacter calcoaceticus	ISRFAWGEV	41 - 49	4-carboxy-muconolactone decarboxylase
Agrobacter tumefaciens	YTRFTWGAP	693 - 701	Beta-glucosidase
Ruminococcus albus	YTQFEISAE	274 - 282	Beta-glucosidase

Alphabetic letters refer to biochemical symbols for amino acids.

In conformity with the new model, it has now been found that sera of BSE affected cattle contain significantly high levels of antibodies to Acinetobacter species.

The present invention therefore provides a diagnostic test for spongiform encephalopathy and other demyelinating conditions in mammals which comprises assaying antibodies present in the mammal which bind to an antigenic peptide which exhibits molecular mimicry of a mammalian myelin peptide, especially one having the sequence FSWGAEGQK. The term "molecular mimicry" refers to a degree of similarity (sequence homology) as between the antigenic peptide and a myelin peptide which results in the formation of antibodies which cross-react with myelin and demyelinate nervous tissue. The presence of such antibodies at elevated levels compared to

those found in unaffected animals is therefore a marker for BSE which may be used to detect BSE at an early stage at which curative or other appropriate action may be taken.

The assay may be carried out using the whole Acinetobacter or other organism as the test antigen. Any strain of Acinetobacter having the antigenic peptide identified above may be used. Alternatively the isolated peptide or a synthetic form of the peptide may be used as antigen. Any suitable type of assay procedure may be used, the ELISA method being especially convenient.

Antibody levels indicative of BSE are those which are significantly higher than the control levels. Usually, levels elevated to about 2 standard deviations above the controls may be taken as a positive indication but margins around this figure may be possible or desirable for purposes of caution.

Procedures for carrying out an assay in accordance with this invention are described in the following illustrative Example, based on comparison of sera from animals known to have had BSE with sera from healthy animals.

MATERIALS AND METHODS

Bovine sera

Sera from 29 animals, which were found at post-mortem to satisfy the criteria of BSE and 18 animals which did not, were supplied by the Central Veterinary Laboratory (CVL) (New Haw, Addlestone, Surrey), an executive agency of the U.K. Ministry of Agriculture, Fisheries and Food (MAFF). The 18 animals which did not have BSE had been referred to CVL because of abnormal behaviour but post-mortem examinations carried out by MAFF had excluded BSE.

Furthermore, 30 sera from animals aged less than 30 months (A<30M) (8 Friesians, 21 Hereford-Friesian and 1 Charolais-Friesian crossbreeds) and 28 sera from animals aged more than 30 months (A>30M) (all dairy Friesians), were used as further controls. These were collected

from a farm, kept under "organic farming" conditions where no case of BSE had been reported. Serum samples were obtained during routine herd testing.

Preparation of bacteria

Acinetobacter calcoaceticus was obtained from the National Collection of Industrial and Marine Bacteria Ltd. NCIMB 10694 (Aberdeen). Cultures were grown in 21 flasks on an orbital shaker for 2 days at 30°C, in 200 ml nutrient broth (Oxoid; 25 g/1). Flasks were inoculated with 10 ml of the corresponding starter culture left shaking at 37°C for 6 hours. Batch culture cells were harvested by centrifugation at 6000 r.p.m. for 20 minutes at 4°C (MSE 18,6 x 250 ml rotor). The pellets of cells were then washed three times with 0.15 M phosphate-buffered saline (PBS; pH 7.4) before being finally resuspended in 20 ml of PBS. A stock solution of the suspension was prepared by diluting in 0.05 M carbonate buffer (pH 9.6) to give an optical density (OD) reading of 0.25 on the spectrophotometer (Corning Model 258).

Enzyme-linked immunosorbent assay

ELISA assays were carried out in the conventional manner. Briefly ELISA plates were coated with bacteria overnight at 4°C and the non-specific sites blocked with PBS containing 0.1% Tween, 0.2% ovalbumin (Sigma, Grade III), plates washed and a 1/200 dilution of test or control serum added. The plates were incubated at 37°C for 1 hour, washed and rabbit anti-cow immunoglobulin (IgG + IgA + IgM) (1:4000) (Dako Ltd.) added. The plates were reincubated for 2 hours, washed and substrate added. The reaction was stopped with a 2 mg/ml solution of sodium fluoride (Sigma). The plates were read at 630 nm on a microtitre plate reader (Dynatech MR 600) and results expressed as OD ± S.E. All studies were carried out under code in that the tester did not know which were test or control sera. The mean OD units of total immunoglobulin antibodies in different groups were compared using Student's t-test.

ELISA METHOD SHEET

1. Dilute antigen in coating buffer, add 200μl to each well. Incubate overnight at 4°C wrapped in foil.
2. Wash out the antigen, using washing/incubation buffer; the wells of the tray should be completely full during the washing stages as the Tween-20 prevents any further protein from being absorbed onto the plastic. Wash 3 times, leaving for approx. 4 minute intervals at room temperature.
3. Incubate the plate at 37°C for 1hr with 0.2% Ovalbumin in washing/incubation buffer.
4. Add 200μl of test serum. Dilutions are made in washing/incubation buffer. Incubate for 2 hours at 37°C wrapped in foil.
5. Repeat washing process as in 2.
6. Add 200μl Horseradish peroxide HRP-conjugated second antibody, also diluted in washing/incubation buffer.
7. Repeat washing process as in 2.
8. Add 200μl substrate (ABTS) to wells; leave to develop colour for approx. 20 minutes in the dark at room temperature. Stop reaction with 100μl of stopping solution and read plate at 630nm.

RESULTS

Antibodies to A. calcoaceticus of total immunoglobulin (IgG + IgA + IgM) were significantly elevated in the BSE sera (mean ± SE: 0.99 ± 0.05) when compared to CVL controls (0.65 ± 0.06) ($t = 4.48$, $p<0.001$), organic farming controls aged more than 30 months (0.57 ± 0.03) ($t = 7.19$, $p<0.001$) and organic farming controls aged less than 30 months (0.53 ± 0.02) ($t = 8.64$, $p<0.001$). These results are shown in the attached Figure.

Legend to figure:

Antibody titres (bar = mean) for 30 controls aged less than 30 months (A<30m), 28 controls aged more than 30 months (A>30m), 18 controls from the Central Veterinary Laboratory (CVL) compared to 29 BSE sera, when tested against Acinetobacter calcoaceticus (Figure la) and E.coli (Figure lb). (Dashed line represents 95% confidence limits for mean of controls: A<30m + A>30m - one tailed test) (OD = optical density).

There was no significant difference between the CVL controls and the organic farming controls aged more than 30 months, but there was a small, statistically significant difference with the sera from animals aged less than 30 months ($t = 2.41$, $p < 0.05$). A re-examination of the CSL control serum with the highest anti-Acinetobacter level of 1.16 OD, showed that it came from a clinically normal control animal, diagnosed as negative to BSE on the statutory diagnostic criteria, and it was also negative when tested for scrapie associated fibrils. This case did however have white matter vacuolation of the substantia nigra and internal capsule, although this had been seen before and not considered significant.

One clear result from these studies, is that in at least in one "transmissible spongiform encephalopathy" (TSE), namely BSE, a specific immune response can be demonstrated against a microbe that is found readily in the environment of cattle and which also happens to possess a molecular sequence resembling bovine myelin.

Other forms of spongiform encephalopathy including Creuzfeld Jacob disease (CJD) and Multiple Sclerosis (MS) are open to explanation on the same model as indicated for BSE. CJD sera and MS sera are currently under test to confirm the presence of cross-reacting antibodies.

CLAIMS

1. A diagnostic test for spongiform encephalopathy and other demyelinating conditions in mammals which comprises assaying antibodies present in the mammal which bind to an antigenic peptide which exhibits molecular mimicry of a mammalian myelin peptide.

2. A test according to Claim 1, in which the mammalian myelin peptide has the sequence FSWGAEGQK.

3. A test according to Claim 1 or 2, for BSE in cattle.

4. A test according to Claim 3, using as the test antigen whole bacteria of an Acinetobacter, Agrobacterium, or Ruminococcus species.

5. A test according to Claim 4, using bacteria of the species Acinetobacter calcoaceticus, Agrobacterium tumefaciens, or Ruminococcus albus.

6. A test according to Claim 3, using as the test antigen a peptide derived from bacteria specified in Claim 4 or 5.

7. A test according to Claim 6, using a peptide of sequence ISRFAWGEV, YTRFTWGAP, or YTQFEISAE.

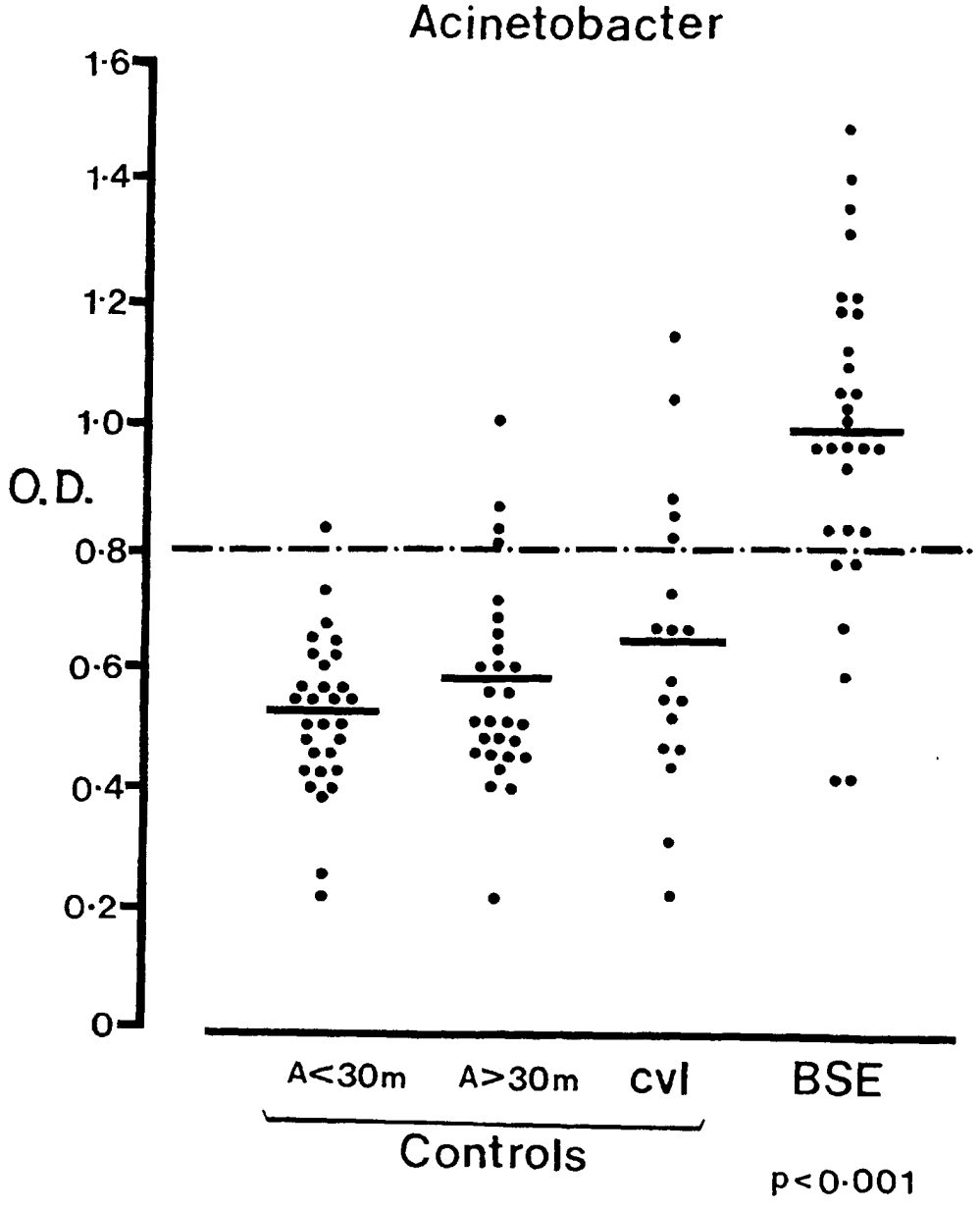
8. A test according to Claim 6 or 7, in which the peptide used is a synthetic peptide.

9. A method of testing for BSE in cattle which comprises assaying sera collected from the cattle for antibodies to a species of Acinetobacter, Agrobacterium or Ruminococcus, or a peptide having a sequence present in said species which mimics a peptide of bovine myelin and identifying animals having a level of antibodies at least about two standard deviations above that of healthy control animals.

10. A method according to claim 9, in which the bovine myelin peptide has the sequence FSWGAEGQK.

11. A diagnostic test kit for BSE in cattle comprising as test antigen a species of Acinetobacter, Agrobacterium or Ruminococcus, or a peptide having a sequence present in said species which mimics a peptide of bovine myelin.

12. A test kit according to claim 11, in which the test antigen is a peptide which mimics the sequence FSWGAEGQK.


Acinetobacter
1·6
1·4
1·2
1·0
O.D.
0·8
0·6
0·4
0·2
0
A<30m
A>30m
cvl
BSE
Controls
p<0·001